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PATENT

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Response Under 37 C.F.R. § 1.116 Expedited Procedure

In re application of: Giovannoni and Connon Art Unit: 1656

Application No. 09/675,382

Filed: September 29, 2000

For: HIGH-THROUGHPUT MICROBIAL CULTURING

Examiner: Alexander H. Spiegler

Date: January 15, 2002

BOX AF
COMMISSIONER FOR PATENTS
Washington, DC 20231

DECLARATION OF Dr. STEPHEN J. GIOVANNONI AND STEPHANIE A. CONNON UNDER 37 C.F.R. § 1.132

We, STEPHEN J. GIOVANNONI, Ph.D., and STEPHANIE A. CONNON, declare as follows.

1. We are co-inventors of and have each read and understand U.S. Patent Application No. 09/675,382 entitled HIGH-THROUGHPUT MICROBIAL CULTURING, and all amendments of that application through today's date. In addition, we have read and understand the following publications. Jovanovich (USPN 5,756,304); Sosnowski *et al.* (USPN 6,051,380); Hoover *et al.* (Hoover, D. and Steenson, L., eds. *Bacterocins of lactic acid bacteria*. San Diego: Academic Press, Inc.; 1993); Cleveland (USPN 4,427,415); and Chee *et al.* (USPN 5,861,242).

2. Copies of our *curriculum vitae* are attached hereto as **Exhibits A and B**. At present, I, Stephen J. Giovannoni, hold an academic position as Professor of Microbiology at Oregon State University and I, Stephanie A. Connon, hold a position as a doctoral candidate in the Department of Microbiology at Oregon State University.

3. We have read and understand a set of proposed amended claims attached as **Exhibit C**, that are to be filed with this Declaration.

4. It is our experience, as researchers well versed in the study of microorganisms, and it is generally believed within the field of microbiology, that less than 1% of the earth's microbial life has been grown using isolation techniques in common use at the time of filing of the present application (see for instance, Hugenholtz *et al.*, *Microbiol. Rev.* 180:4765-4774, 1998, a copy of which is attached hereto as **Exhibit D**). Only about half of the 40+ known prokaryotic phyla have laboratory-cultured representatives, and new uncultured phyla are continually being discovered. This has long been recognized by others of ordinary skill in the art (see for instance, Bull *et al.*, *Microbiol. Mol. Biol. Rev* 64:573-606, 2000, a copy of which is attached hereto as **Exhibit E**), who state that "...the numbers of prokaryotes which can be readily cultivated from environmental samples is only a small and skewed fraction of the diversity present. The inability to cultivate even the most numerous microorganisms from natural habitats has been referred to as the 'great plate count anomaly.'" (Bull *et al.*, page 580, col. 1 lines 59-64).

5. Our invention, as defined by the claims of **Exhibit C**, provides methods that can detect cell densities that are far lower than the lower limits of detection available using optical density readings. Using our methods, one is able to detect a culture with a concentration of cells as low as 1 x 10^5 cells/milliliter whereas the lower limit of cell detection by optical density readings is 1 x 10^6 cells/milliliter (see footnote 11, page 103; Atlas, R. ed. *Microbiology: fundamentals and applications* New York: Macmillan Publishing Company; 1988; attached hereto as **Exhibit F**). Our methods are capable of easily detecting as few as 200 cells in a growth chamber, which equates to seven to eight divisions from a single starting cell. None of the low-density cultures detected with our invention would have been detected using optical density measurements; our invention redefines what can be considered a "culture" of a microorganism.

6 Our claimed methods address a long-standing problem inherent in the standard microbiological culturing techniques. This problem is emphasized in Bull *et al.* (2000) which disclose that "...spectacular patterns of prokaryotic diversity had gone undetected using standard cultural and characterization procedures" (Bull *et al.*, page 580, col. 1 lines 56-58). Using our claimed methods, up to 14% of the cells gathered from coastal water and 20% of the cells gathered from groundwater have been cultured, which is a 100- to 10,000-fold increase in the culturability of microorganisms from these environments. As a result, we have detected approximately 600 cultures with our invention, many of which are unique cell lineages that will be named as new species and genera by microbial systematists.

7. Our claimed methods isolate microorganisms directly from natural environments using automated techniques and high-throughput methods. In addition, our methods use cell arrays wherein unidentified whole microorganism cells are deposited on a solid surface and known probes are washed over the cells. The cells are in this manner identified based on the ability or inability of the probes to bind to the arrayed cells. The combination of the low cell density detection, high-throughput and cell array methods enables screening and identification of hundreds of microorganisms, which have not been previously cultured using standard culturing techniques.

8. One particularly important result obtained using our invention is the successful isolation and propagation of SAR11, one of the most abundant microorganisms on the planet but which was previously never isolated and grown in culture. SAR11 accounts for 26% of all ribosomal RNA genes detected in seawater, and has been found in every pelagic marine bacterioplankton community tested by this invention. SAR11 is representative of a pervasive problem in microbiology: despite its ubiquity in nature, it has defied cultivation efforts. The isolation of SAR11 is described in our manuscript, *Cultivation Of The Ubiquitous Marine Bacterium SAR11* (attached hereto as **Exhibit G**).

9. We believe there are several fundamental differences between the teachings of Jovanovich and our invention. First, Jovanovich isolates and cultivates microbes from the environment

using standard microbial techniques, then *screens* them with his invention. In stark contrast, our methods employ novel high-throughput methods in order to *isolate* the microorganisms from nature, which could not be isolated using standard cultivation techniques. Jovanovich relies on optical density readings to detect microorganisms; reading optical density is too insensitive a detection method to detect the cells in the cultures isolated using our claimed methods. Finally, Jovanovich uses standard isolation techniques to isolate microorganisms and then screens them for their bioremediation capabilities, discarding all microorganisms that do not exhibit such capability. Jovanovich therefore cultivates and identifies only a very small percentage of the cells in the starting sample, as compared to our methods that optimize detection of a broad spectrum of microorganisms. The Jovanovich disclosure is thus limited by the same isolation practices that our methods are expressly designed to overcome. The unique and new organisms we have isolated using our methods would never have been detected, let alone isolated, by the methods disclosed in Jovanovich.

10. Our methods are further unique and non-obvious in the use of cell arrays to identify cultures phylogenetically using nucleic acid hybridization. The microarrays disclosed by Chee *et al* differ fundamentally from our methods of using cell arrays. The Chee *et al.* microarrays consist of known probe DNA deposited on a solid surface, which is then washed with unknown DNA in order to determine a genetic characteristic of the unknown DNA. There are at least three fundamental differences between the cell array system we use in our claimed methods and the Chee *et al* microarray system: 1) we are depositing whole cells onto the array and not purified DNA; 2) we use the arrays for counting cells, and 3) we are hybridizing many cells to a single probe rather than many probes to DNA from a single cell.

11. It is our understanding and belief, as experts in the field, that our invention as it is currently claimed is distinct from the subject matter disclosed in Chee *et al* and Jovanovich. No teaching is provided by Jovanovich or Chee *et al.* that would enable one of ordinary skill in the art to isolate the diversity of microorganisms isolated by our invention or detect microorganisms at the cell densities detected by our invention. Additionally, there is no implicit teaching in any of the cited

references, or any combination of these references, that would have led one of ordinary skill in the art to the methods we are claiming.

12. All statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true. Further, these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Date: Jan 15, 2002

Stephen J. Giovannoni, Ph.D.

Date: Jan 15, 2002

Stephanie A Connon